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CITATION:

Hishida, Mafumi ...[et al]. 13. Spontaneous formation of phospholipid giant vesicles(poster presentation,Soft Matter as Structured Materials). 物性研究 2005, 84(6): 892-893

ISSUE DATE:

2005-09-20

URL:

http://hdl.handle.net/2433/110315

Spontaneous formation of phospholipid giant vesicles

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生体膜を主に形づくるリン脂質は、水中でベシクル構造をとる。特に大きさが 1 μm を超えるものを giant vesicle (GV) といい、細胞膜モデルなどとして盛んに応用されている。GV を効率的に生成する方法の一つとして静置水和法がよく知られているが、この方法でなぜ自発的に GV が形成されるのか、そのメカニズムは明らかではなかった。本研究では、静置水和法で用いられるリン脂質 Dry Film の構造に着目し、相の違いによる形態の違いと GV 形成に与える影響を調べた。これにより、GV の形成メカニズムに関して重要な知見を得た。

1 Introduction

Phospholipid molecules, which are the main constituent of cell membrane, form micelles or vesicles in water. Among these colloidal suspensions, giant vesicle (GV) has been actively investigated as a cell model or a micro-reactor, because the size of GV is comparable with the usual size of living cells; 1-100 μm. For effective generation of GV, several methods have been developed such as the electro-formation method or the solvent-evaporation method. However, most of these methods are not suitable for biological experiments due to physical stresses or harmful chemicals used in the procedure. On the other hand, the natural swelling method [1] has no such unsuitable elements, where GVs are spontaneously prepared by hydrating dry phospholipid film. Although this method has been well known for long time, the mechanism of GV formation has not been fully understood. Therefore, preparation of GV is still depending on technical skills or individual experiences.

To clarify the mechanism of GV formation, it is important to observe the structure of dry phospholipid film and the morphology of GV, and investigate the relation between them. Especially, we notice morphological change of dry phospholipid film that depends on the phases below and above the main phase transition temperature, i.e., the ordered gel phase and the disordered liquid-crystalline phase.

2 Results and discussion

The morphologies of dry DOPC and DPPC films were observed by phase-contrast microscopy and atomic force microscopy (AFM). Dry DOPC film in the liquid-crystalline phase had smooth

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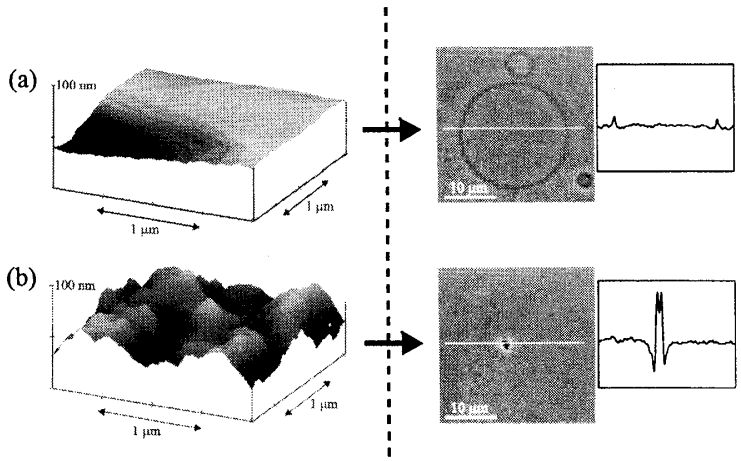

Figure 1: (Left) AFM images of dry phospholipid films. (Right) Hydrated suspensions observed by phase-contrast microscopy and cross-section profiles along the latitudinal lines. (a) DOPC. (b) DPPC. Both were observed at room temperature.

surface exhibiting many steps, while dry DPPC film in the gel phase had rather rough surface (Fig. 1 (Left)). Once the DPPC film was annealed above the main transition temperature, the film had a terrace-like morphology similar to the morphology of the DOPC. These results indicate that the morphology of dry phospholipid film depends on its phase.

Next, we hydrated these dry films by adding excess water at room temperature. By hydrating the DOPC film, many GVs were formed effectively. On the other hand, no GVs were obtained by hydrating DPPC film that had rough surface morphology. Hydration of the annealed DPPC film gave no GVs. These results indicate that both the terrace morphology and the large membrane fluctuation are necessary for the GV formation. This discussion is consistent with the result by Lasic, where the size of vesicle depends on the topology of the substrate [2].

3 Conclusion

The two points are concluded. (i) Dry phospholipid film in liquid-crystalline phase is regularly stacked, whereas that of gel phase depends on the preparation process. (ii) The terrace-like morphology and adequate membrane fluctuation in water are essential to generate GVs effectively [3].

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